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Three Dimensional Analysis of Sea Urchin Mitotic Apparatus with SFP Method

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Visualization of cytoskeleton in sea urchin embryos is an important process for its developmental analysis on biology. There are many suggestions between the early developmental processes (i.e. nuclear positioning, formation of asymmetric mitotic apparatus and determination of division plane). Despite of the existence of these suggestions, it was very hard to visualize cytoskeleton in whole mounted embryo. One difficulty of visualization was the relatively big diameter of sea urchin egg. The big diameter of sea urchin egg interferes to the normal working distance of conventional immuno fluorescence light microscopy and results low resolution of image unless samples were sectioned or flattened artificially. In fact, most objective lenses with high magnification for conventional fluorescence microscopy have about sixty to eighty μ meter of working distance. So, the whole diameter of sea urchin egg comes to physical limit of objective working distance. Three dimensional visualization of embedded physical sectioned eggs requires very large number of sections. When these images are exported to the computer system for three dimensional visualization, it needs more power of processor and more large area of memories and hard disk for to make the reconstructed image of spatial distribution of cytoskeleton. Optical sliced images are gotten easily without physical sectioning and exported directly to the reconstruction program. One problem of three dimensional reconstruction is the resolution of resulted image. Usually, final 3-D image showed low resolution and "CG like" artificially enhanced view. The solution for this problem is to increase the number of optical sections, which means longer LASER excitation to the samples. To minimize the fading of fluorescence dye, more fast scanning method is required for high resolutional three dimensional analysis. Another problem is the way of expression of the three dimensional objects. The most popular style of visualization is stereo-pair or red-green stereo images. These visualization methods are easy to use but does not show the depth of samples naturally. So, I choose the Simulated Fluorescence Process (SFP) method for the expression of reconstructed three dimensional images. This process makes a rendered realistic image with ray tracing algorithm after the correction of axial distortion. The result of ray tracing is more natural 3-D view than stereo (or red-green) type reconstructions. Cytoskeletons in the mitotic phase embryos were preserved by the optimized isolation medium and fixed. After immuno-stained, confocal images were obtained by the InSIGHT plus-IQ (Meridian Instruments Inc.) equipped on the inverted microscope (IMT-2, Olympus). Embryos were settled on the grass-bottom culture dish and observed with 60X objective (Plan-Apo, NA=1.4). Optical series of sectioned confocal images were obtained for each 0.1micro meter or 0.5 μ meter in depth, and for 100 to 256 in total number. As a result, whole height of mitotic apparatus or embryo is sliced in the confocal sections. Images were analyzed with three dimensional SFP algorithm equipped in InSIGHT plus-IQ system, or exported to the IRIS Indigo (Silicongraphics, Inc.) and rendered with VoxelView system (Vital Images Inc.). Reconstructed SFP images of first mitosis were made through the metapahse to late telophase embryos and streak stake embryos. Projected X-Y image and computer-aided X-Z sectioned images were also made for the whole height of embryos. Animated series of SFP images were also made for the analysis from multi-pointed view.

This work showed the possibility of three dimensional analysis of microtubules in sea urchin embryo with SFP, projection and X-Z sectioning method. With SFP method and enough number of optical sliced images, reconstructed images are free from

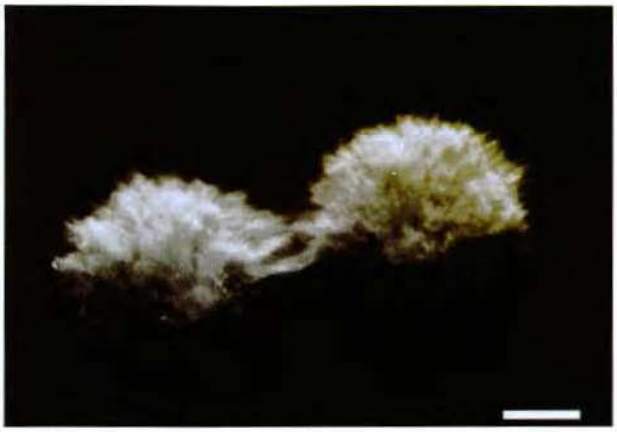

Fig. 1. SFP reconstructed mitotic apparatus of sea urchin *Hemicentrotus pulcherrimus* at the first metaphase. Whole reconstructed mitotic aster and spindle are clearly shown. (Bar=10 μ meter)

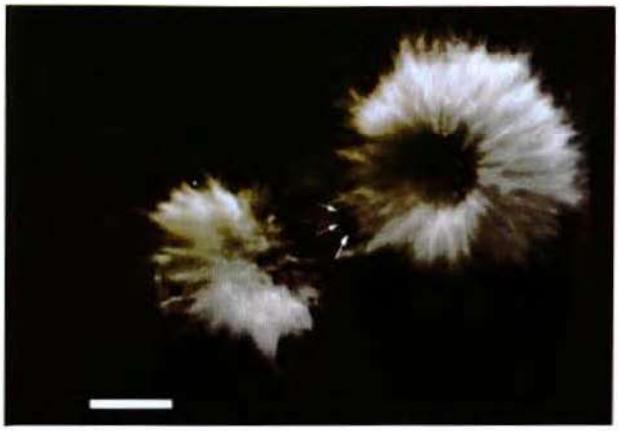

Fig. 2. SFP reconstructed image at the anaphase embryo. Arrows indicated the position of chromosome in the mitotic spindle. (Bar= 10μ meter)

artificial enhancement of microtubule image in the whole height of embryos. These results suggest the usefulness of confocal system for the analysis of the three dimensional distribution of cytoskeleton.